ECC preparation kit

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Product components

Components	For preparation of 40 competent vials, Cat. No. S-8290
A streak plate of *E. coli* DH5α bacteria	1 plate
ECC growth medium	5 ml
ECC enriched medium	100 ml
ECC1 buffer	45 ml
ECC2 buffer	2.5 ml
ECC3 buffer	25 µl

Product description

The Enhanced Competent Cell (ECC) Preparation Kit and buffer set provide a simple and efficient method for preparing chemically competent *E. coli* cells for DNA transformation. The procedure is quick, taking less than 45 minutes to produce reliable chemically competent cells. The transformation efficiencies are high, typically achieving very high number transformants with most *E. coli* strains. The kit includes all necessary buffers and medium to generate 2 ml of competent cells (Approximately 40 vials, each with a volume of 50 microliters).

Protocol for preparation of competent cells

Day 0: PREPARE A FRESHLY STREAKED PLATE OF THE BACTERIA OF INTEREST.

1. The plate provided in the kit is freshly streaked and can be used for making competent cells upon receiving. In this case, follow the procedure from step 3.
2. If the procedure for making competent cells will not be started right away, keep the kit components at 4°C. To keep the culture of bacteria fresh, re-streak the bacteria on a new agar plate (LB agar plate without antibiotic), and after overnight growth at 37°C, keep the plate up until 10 days at 4°C.

   Note: It is strongly recommended to always use the fresh streak of bacteria. Do not use bacterial cells that are kept at 4°C for more than 10 days.

Day 1: PREPARE STARTING CULTURE OF BACTERIA

3. To start a small culture of bacteria, pick a single colony from a freshly streaked plate.
4. Inoculate the colony into 5 ml of the ECC growth medium in a sterile 15-ml Falcon tube.
5. Incubate the culture 15 hrs at 37°C with shaking at 120-150 rpm (start at 6 p.m.).

Day 2: PREPARE COMPETENT CELLS

6. Add the entire content of buffer ECC3 to buffer ECC2.
7. Inoculate 100 ml of the ECC Enriched medium in a baffled Erlenmeyer flask with 0.5 ml of the overnight culture from step 5.
8. Incubate at 37ºC with shaking at 180-220 rpm.
9. When the optical density of the bacterial culture at 600 nm (A600) reaches a value between 0.4 and 0.5, place the culture flask on ice and chill for 5-10 minutes.
10. Transfer the culture to pre-chilled sterile 50-ml polypropylene centrifuge tubes.
11. Centrifuge the tubes at 5000g for 5 minutes at 4°C to pellet the bacterial cells. Carefully discard the supernatant and keep the pellet.
12. Genttly resuspend the cell pellet in 2 ml of ice-cold ECC1 buffer. Add an additional 18 ml of ECC1 buffer to reach a total of 20 ml in a 50 ml tube.

    Note: It is important not to vortex the cells at this stage and to keep them on ice while resuspending.
13. Incubate the tube of resuspended cells on ice for a period of 15 minutes.
14. Centrifuge the tubes at 2000g for 10 minutes at 4°C to pellet the bacterial cells.
15. Carefully discard the supernatant and keep the pellet. Genttly resuspend the cell pellet in 1 ml of ice-cold ECC2 buffer.

    Note: It is important not to vortex the cells at this stage and to keep them on ice while resuspending.
16. Place 40-45 screw-capped tubes with conical bottoms on ice. Aliquote the cell suspension into 50 µl portions in these tubes.
17. Immidiately place the tubes in a -80ºC freezer. Alternatively, the tubes can be flash-frozen in a dry ice-ethanol bath, and then be transferred to a -80°C freezer. ■